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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER JBP-ONR- 3	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
TITLE (and Subtitle) MICROWAVE IRRADIATION AND COLD EXPOSURE		5. TYPE OF REPORT & PERIOD COVERED FINAL 7/18/84 - 7/17/85
		6. PERFORMING ORG. REPORT NUMBER
AUTHOR(s) Eleanor R. Adair		8. CONTRACT OR GRANT NUMBER(s) N00014-84-C-0535
PERFORMING ORGANIZATION NAME AND ADDRESS John B. Pierce Foundation Laboratory 290 Congress Avenue New Haven, CT 06519		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
CONTROLLING OFFICE NAME AND ADDRESS Dept. of the Navy-Office of Naval Research Physiology Program: Code 441CB Arlington, VA 22217		12. REPORT DATE August 17, 1988
		13. NUMBER OF PAGES 9
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report)
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Unlimited

DTIC ELECTE AUG 2 5 1988 S D H

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Squirrel monkey, cold exposure, 2450-MHz CW microwaves, metabolic heat production, behavioral thermoregulation

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A pilot study investigated the consequences of chronic exposure (40 h/wk) to 2450-MHz CW microwaves, or sham exposure, in a cold (18 °C) environment on the thermoregulatory responses, both behavioral and physiological, of squirrel monkeys. Four animals exposed to microwaves at 20 mW/cm^2 exhibited responses that indicated slight amelioration of the mild cold stress while four sham-exposed animals sustained no thermoregulatory deficits. Although (please see reverse side of page)

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S/N 0102-LF-014-6601

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the sample size was too small to yield statistically reliable data, general trends in the results support the view that exposure to microwaves of moderate intensity in the work environment is benign in terms of long-term effects on thermoregulatory processes.

MICROWAVE IRRADIATION AND COLD EXPOSURE

Eleanor R. Adair

John B. Pierce Foundation Laboratory, Inc.
290 Congress Avenue
New Haven, CT 06519

August, 1988

<u>FINAL REPORT</u>

ONR Contract N00014-84-C-0535

Prepared For

Office of Naval Research
Department of the Navy
800 N. Quincy Street
Arlington, Virginia 22217

MICROWAVE IRRADIATION AND COLD EXPOSURE

I. INTRODUCTION

Electromagnetic radiation of the microwave frequency range is energy that can, under appropriate conditions, produce heating of both peripheral and deep body tissues. Although the current ANSI voluntary standard (ANSI, 1982) for safe continuous exposure to microwaves is below the threshold for cutaneous warmth sensation in man, other thermal and athermal consequences of exposure to low energy levels (e.g., 1-10 mW/cm2) are largely unknown. In particular, weeks or months of exposure to low-energy microwaves can be potentially stressful, especially if the ambient temperature is at or above thermoneutrality, and may produce subtle adaptive changes in bodily function (Goldman, 1983). Equally-subtle changes may occur if chronic low-level microwave exposure is superimposed on an ambient temperature that is below thermoneutrality. In the latter case, however, the adaptive changes may benefit the organism because the exogenously-supplied energy can replace that produced in the body through metabolic processes (Adair and Adams, 1982).

Some of the fundamental response systems in the body that can potentially be altered by long-term exposure to a source of microwave heating are those that function to regulate the body temperature. These involve both autonomic (heat production and heat loss) and behavioral adjustments. Thermoregulatory responses of both types, together with their underlying neural control mechanisms, are well understood in a wide variety of species, including man. Animal studies of the consequences of exposure to low-intensity microwaves in cool environments should be designed to probe several basic questions: 1) What are the effects on normal thermoregulation, both physiological and behavioral, of chronic exposure to a cool environment in combination with a microwave source of moderate intensity? 2) Can chronic exposure to microwaves be demonstrated to benefit the exposed animals in the sense that deleterious consequences of cold exposure are ameliorated? 3) Will any deleterious effects of microwaves *per se* occur during chronic exposure when the rate of energy absorption is of the order of the resting metabolic heat production of the organism? The research described below was designed to provide answers to these basic questions, using the squirrel monkey as an animal model.

Since human subjects cannot be experimentally exposed to microwaves, the potential hazards and benefits of such exposure, both thermal and athermal, cannot be assessed directly. Other methods must be found. Clinical evaluation is unsatisfactory because the complex and variable nature of the exposure conditions cannot be quantified. We and other research teams have demonstrated repeatedly that man's

nearest relatives, non-human primates, can provide the necessary data on the biological effects of microwaves under highly controlled experimental conditions; the use of appropriate dosimetry and scaling procedures then allow extrapolation of observed effects so as to characterize the implicated hazard or benefit for humans.

In the experiments described below, the homogeneity of the microwave field was fully characterized and the frequency, polarization, intensity, and duration of each exposure was precisely controlled. The ambient temperature was closely regulated so that the possible synergistic action of this variable with microwave energy could be evaluated. Under the assumption that microwaves can have a thermal component and with the knowledge that any thermal aspect of the environment has the potential for thermoregulatory consequences, the total thermoregulatory response of the animal subject was quantified. To do this, we utilized standardized behavioral and physiological response measures and specialized instrumentation adapted for use in microwave fields.

BACKGROUND AND SUPPORTING DATA

The experiments conducted in this project were designed to dovetail with several other projects in our laboratories, ongoing for several years, that have been probing the thermoregulatory consequences of acute and chronic exposure of squirrel monkeys and other mammals to microwave radiation. In particular, a four-year project examined changes in behavioral and physiological thermoregulation during and after 15-week exposure of squirrel monkeys (8 h/day, 5 days/week) to low intensity microwave fields (2450 MHz CW at 1 and 5 mW/cm2) at controlled environmental temperatures of 25, 30 and 35 °C. This study was conducted against a background of detailed knowledge of thermoregulation in this species (Adair, 1985). In general, the results of the study were largely negative, pointing up the extreme adaptability of the squirrel monkey subjects when exposed to very low intensity microwave fields in thermoneutral environments (Adair, et al., 1985).

At the same time, data from another project being conducted in our laboratories suggested that long term microwave exposure in <u>cool</u> rather than warm environments may produce an amelioration of cold acclimatization. Infant rats (aged 6 to 16 days) were exposed four or eight hours daily to 2450 MHz CW microwaves at a power density of 5 mW/cm2 and an ambient temperature of 30 °C (cool for rat neonates). Other rats were sham exposed at the same environmental temperature. Tests of physiological thermoregulatory capacity at the end of the chronic exposure period indicated that the microwave-exposed animals responded metabolically more like normal animals that had been left with the mother than did their

sham-exposed counterparts. This was particularly evident if the animals were fed partway through the daily exposure period so as to reduce weight loss during the time away from the dam.

To extend this preliminary finding, we conducted a pilot study in which we subjected four squirrel monkeys to chronic microwave (or sham) exposure in a cool (18 °C) environment, 40 h/wk for 15 weeks. Power density was held constant at 5 mW/cm2. During the course of chronic exposure, each animal was administered 7 tests to assess thermoregulatory function, 4 physiological tests and 3 behavioral tests. Each type of test involved standardized protocols. The behavioral test, 240 min in duration, permitted the animal to select it's preferred environmental temperature (Ta) by choosing between two available air temperatures, 10 and 50 °C. In addition to the measure of Ta selected, colonic temperature and four representative skin temperatures (abdomen, tail, leg, foot) were also measured continuously. The physiological test involved a series of Ta challenges to 20, 26.6, 29.9, 33.3, and 36.6 °C to assess levels of heat production and heat loss responses at each Ta. Measured variables included metabolic heat production (calculated from oxygen consumption), colonic temperature, four representative skin temperatures (abdomen, tail, leg, foot), and sweating rate from the foot. Whole-body thermal conductance was assessed from the core-to-ambient temperature gradient.

The data from individual tests on each animal were compared with extensive pre-chronic exposure data. During an 8-week post-exposure follow-up period, 2 additional tests of each type were conducted on each animal. Since the number of animals in each treatment group was limited to two, no statistical analyses could be performed on the data. Trends in the data indicated that the cold-exposed animals concomitantly exposed to microwaves derived clear benefit from the exogenously-supplied energy which was, at 5 mW/cm2, the equivalent of about 15% of the resting metabolic heat production. They maintained a normal deep body temperature by vasoconstricting the tail while maintaining an otherwise normal skin temperature, and by strategic adjustments in metabolic heat production. On the other hand, thermoregulation in the sham-exposed animals appeared to have been compromised, not only during the 15 weeks of chronic cold exposure but also throughout the 8-week follow-up period. The latter was particularly evident in the data from the behavioral tests during which these animals selected a Ta 1 to 2 °C warmer than their normal (baseline) preference in order to achieve a normal colonic temperature.

METHODS AND PROCEDURE

The present project was designed to further examine the effects of chronic exposure to 2450 MHz CW microwaves, at a

controlled cool (18 °C) environmental temperature, on the physiological and behavioral thermoregulatory responses of squirrel monkeys. Groups of four animals, two microwave-exposed and two sham-exposed at the same environmental temperature, underwent chronic exposure 8 hours/day, 5 days/week for 15 weeks. During this treatment period, all animals were administered 4 standard physiological tests and 3 standard behavioral tests to assess a wide variety of thermoregulatory responses to environmental challenge. These test results were compared with extensive pre-exposure baseline data derived from similar experimental protocols and 2 post-exposure follow-up tests of each type.

This experimental protocol was conducted twice with the microwave power density set at 20 mW/cm2. On the basis of our dosimetry, this level represented a whole-body SAR of 3 W/kg, about 80% of the resting metabolic heat production of the squirrel monkey. We believed that this level would confer all of the advantages of moderating the effects of cold exposure that were conferred by 5 mW/cm2 during the pilot study, while also affording an opportunity to produce deleterious thermoregulatory consequences. The exposure system and dosimetry, exposure chambers and cages, and the test systems for both the physiological and behavioral tests have been described in detail by Adair, et al., (1985).

A. Treatment of Individual Groups

During the 15-week exposure period, the monkeys occupied the exposure (or sham exposure) chambers from 0830 to 1630 hrs daily, five days a week. At the end of each exposure day, they were returned to their individual cages in the vivarium and were fed their daily food ration.

Prior to the chronic exposure phase, we conducted a pre-exposure phase during which each animal underwent 4 standard physiological tests and 4 standard behavioral tests. During the chronic exposure phase, each animal underwent 4 standard physiological tests (beginning in the 3rd week of chronic exposure) and 3 standard behavioral tests (beginning in the 5th week of chronic exposure). Following the 15-week chronic exposure phase (post-exposure follow-up), each animal underwent 2 tests of each type.

B. Detailed Protocols for Individual Tests

1. Standard Physiological Test

Monkeys were tested individually in the physiological test chamber (Adair, et al., 1985). They were chair restrained so that the following physiological variables could be measured: colonic temperature, four representative skin temperatures (abdomen, tail, leg, foot), metabolic heat production, and, on some occasions, evaporative water loss from the foot. The four skin temperatures were used to

calculate a weighted mean skin temperature according to the relation (Stitt, Hardy & Nadel, 1971):

$$\overline{T}sk = 0.45\ Tabdomen + 0.37\ Tleg + 0.11\ Ttail + 0.07\ Tfoot.$$

Individual temperatures of the tail and foot were also analyzed for evidence of changes in vasomotor state. A hood over the animal's head collected the expired air which was drawn outside the chamber at 7 L/min and analyzed for oxygen content. Metabolic heat production (M) was calculated from oxygen consumption assuming an RQ of 0.83. Rate of evaporative water loss was indexed by the change in dew point temperature (Tdp) of air drawn through a Plexiglas boot enclosing the animal's right foot.

During the test, ambient temperature (Ta) was rigorously controlled and varied in a prescribed manner. At the start of the test, Ta was set at 20 °C which continued for a minimum of 90 minutes or until the animal was fully equilibrated. Step changes in Ta to 26.6, 29.9, and 33.3 °C followed in order, sufficient time being allowed at each new Ta for a physiological steady state to be reached. Throughout each test, each physiological variable was monitored once a minute by an on-line computer, displayed on an on-line plotter, and stored on disk for subsequent statistical analysis.

Four such tests were conducted on each animal during the weeks preceding the initiation of chronic exposure. These baseline data were averaged to provide individual baseline functions against which the results of subsequent tests, conducted during and after chronic exposure, were compared.

2. Standard Behavioral Test

The behavioral test was designed to reveal two aspects of environmental preference: 1) the ambient and body temperaturess normally preferred and 2) the modification of this preference that may have been produced by chronic exposure to a controlled microwave field in a cold environment.

Monkeys were tested individually in a behavioral test chamber to determine their preferred ambient temperature. Each animal was highly trained to regulate the temperature of the air that flowed over his body inside the chamber. A valve system allowed air from one of two themostatically-controlled sources to circulate through the test chamber. The monkey was trained to pull a response cord to operate the valves, thereby selecting between two preset air temperatures, 10 and 50 °C. Under long-term exposure, all animals alternated between the two so as to achieve a time-averaged preferred air temperature of 35-36 °C. During the behavioral tests of 240 min. duration, the monkey was chair restrained so that colonic and four representative skin temperatures could be measured in addition to the air

temperature selected by the animal. Each variable was monitored once a minute by an on-line computer, displayed on an on-line plotter, and stored on disk for subsequent analysis.

Four such tests were conducted on each animal during the weeks preceding the period of chronic exposure. The data were averaged to provide individual baseline functions against which the results of subsequent tests, conducted during and after chronic exposure, were compared.

C. Analysis of Data

In general, each animal served as its own control and the data derived from individual tests were compared with the extensive baseline data for that animal. Because the trends of the data from individual tests appeared to be similar, and because the baseline data across animals were similar, the data from all animals under each condition of exposure were also pooled for further analysis.

RESULTS AND DISCUSSION

A. Behavioral Thermoregulation

During the four tests of behavioral thermoregulation conducted prior to the initiation of chronic microwave (or sham) exposure, all monkeys selected an average preferred air temperature of 36 to 36.5 °C. This selection yielded stable skin and deep body temperatures at the levels characteristic for this species (Adair, 1985). The animals sham exposed at a Ta of 18 C continued to select this same average preferred air temperature during the entire period of chronic exposure and the subsequent follow-up period. However, there was a trend, albeit not statistically significant, for those animals chronically exposed to microwaves at 20 mW/cm2 to prefer a slightly cooler environment midway during the chronic exposure phase of the protocol. This was particularly evident during the second of the three behavioral tests; the preferred Ta had fallen by nearly 1 °C. This trend appeared to be reversed at the third test when there was no longer any difference in preferred Ta between sham and microwave exposed animals. During the follow-up period, no significant difference in preferred Ta was measured between sham- and microwave-exposed animals, although the previously-microwave-exposed tended again to select a slightly cooler environment than their sham-exposed counterparts. Thus, the effects of chronic microwave exposure in a cool environment on thermoregulatory behavior were minimal, transitory, and somewhat unpredictable. On the other hand, the chronic exposure of sham animals to an 18 °C environment did not appear to have had deleterious thermoregulatory consequences, at least in terms of the preferred thermal environment which remained essentially the

same throughout. In this respect, the findings of the pilot study described above were not replicated.

Analysis of the regulated colonic temperature and mean skin temperature during behavioral thermoregulation involved pooling of the data from the four animals in each group that were similarly treated. Steady-state levels of each variable were calculated across the last 60 minutes of each behavioral test. Baseline temperature data (prior to chronic exposure) were identical for all animals and showed little subsequent change for those sham-exposed. However, microwave-exposed animals exhibited a lower mean colonic temperature than shams (by about 0.3 °C), both across the period of chronic exposure and during the follow-up period. Since the standard errors were of the same order of magnitude as the difference, the difference is not statistically reliable, but the trend is unmistakable. A similar trend occurred in the mean skin temperature, but the variability in response was higher still, rendering individual differences statistically unreliable. It is clear that, given the variability in response from monkey to monkey, a larger number of animals per group would be required to confirm statistically the trends we have observed.

B. Physiological Thermoregulation

For the pre-exposure phase, the results of the four baseline tests on each animal were averaged. For the chronic exposure and post-exposure follow-up phases, results of individual tests on each animal were compared with the mean baseline values to assess temporal changes. The data collected during the pre-exposure phase were analyzed as if treatment had already occurred to detect possible differences in pre-exposure values. As in our earlier published report (Adair, et al., 1985), reliable differences in baseline data were found only in the mean skin temperature, attributable both to a large variability in normal values for this dependent variable and to the small sample size.

Levels of metabolic heat production (M), colonic temperature (Tco), and mean skin temperature ($\overline{T}$sk) were analyzed, as a function of test temperature, for all the tests conducted during the chronic exposure phase and the post-exposure follow-up phase to determine the potential effects of microwave or sham treatment in the 18 °C environment. Midway in the chronic exposure phase (7 to 12 weeks), M and Tco of sham-exposed animals were reliably higher than microwave-exposed animals in the thermoneutral test environments (26.6, 29.9, and 33.3 °C). The Tsk of sham animals was also higher than microwave-exposed but not reliably so because of the high variability (see above). It is significant, however, that both skin and deep body temperatures as well as metabolic heat production were nearly always higher in sham-exposed than in microwave-exposed animals, whatever the test temperature. At the test

temperatures employed, the primary ongoing physiological thermoregulatory response would be vasodilation, particularly of the tail (Stitt and Hardy, 1971). Our results imply that heat production responses had been suppressed and vasomotor responses had been augmented by microwave treatment in the cool environment. Further, the physiological data support the trend in the behavioral data (see above) that chronically microwave-exposed animals may prefer a slightly cooler environment, at least during the chronic exposure phase.

While these specific trends in response change were evident, no definitive conclusions can be drawn about the effectiveness of microwave exposure in cool environments because of the small sample size of 4 animals per group. Additional replications of the standard protocol would have to be conducted. We can only generalize that the microwave treatment appeared to have produced some amelioration of the thermal stress imposed by the 18 °C environment and some possible adaptation that took the form of a preference for slightly cooler surroundings. On the other hand, the normal responses exhibited by the sham-exposed animals indicated that an 18 °C environment provides minimal thermal stress when imposed only 40 hours/week.

As in our earlier study of the effects of chronic microwave exposure in thermoneutral environments (Adair, et al., 1985), it can be argued that since the monkeys spent less than 25% of their time in the chronic-exposure environment, any alterations in thermoregulatory responses (e.g., a reduction in metabolic heat production as reported by Adair and Adams, 1982) could have been reversed during th hours spent in the home cage. By design, the 40 hour/week exposures simulated the temporal conditions in the workplace rather than in the total living environment. Human beings who work in warm environments acclimatize to heat with as few as 2-4 hours/day exposure for 7-9 days (Goldman, 1983). The microwave-exposed monkeys exhibited a generalized adaptation of this nature that appeared to develop as the chronic exposure phase progressed. The most important conclusion to be drawn from this study, based on the trends in the data, is that the adaptability of the mammalian thermoregulatory system prevents long term disturbances in thermoregulation when microwave exposure, even at moderately high levels, occurs chronically in the work environment.

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